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Evaluation of Pakistani Elite Wheat Germplasm for T1BL.1RS Chromosome Translocation

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ABSTRACT

Rye (*Secale cereale*) chromosome 1RS harbors multiple genes including *Lr26, Sr31, Yr9* and *Pm8* conferring disease resistance and tolerance to abiotic stresses. The introgression of the rye 1R chromosome short arm has enormously contributed to increase of genetic diversity in wheat. Utilization of such translocations in breeding programs demands identification of wheat germplasm possessing the wheat-alien chromosome translocation. This study was designed to screen a set of 102 Pakistani wheat cultivars and candidate lines to identify the rye T1BL.1RS translocation, using cytological, biochemical, and molecular techniques. Results revealed that 12 out of the 40 wheat cultivars were found to have this alien introgression. In the National Uniform Wheat Yield Trials (NUWYT) group, 10 of 23 entries of the rainfed category were identified as carrying 1BL.1RS translocation, while 4 out of 39 genotypes were present in the irrigated category of both NUWYT crop seasons. The valuable information generated can be useful in the crop improvement programs for the production of germplasm possessing T1BL.1RS translocation, in order to enhance the genetic variability in local wheat cultivars and, also, avoid the preponderance of T1BL.1RS candidates.

Keywords: Genetic diversity, Constitutive heterochromatin banding, Marker analysis, Mitotic counts, Rye introgression, Wheat collections.

INTRODUCTON

Food security is a pressing issue globally. The burgeoning world population is estimated to increase to 8.5 billion by the year 2025. It would demand food supplies which are to be doubled to meet the needs (Mujeeb-Kazi and Rajaram, 2002). Among the agricultural crops, wheat is of utmost importance as it is a source of feeding the major proportion of the world population. Wheat improvement, like any other crop, has relied on genetic diversity for enhancing its productivity. Several types of genetic resources such as landraces, wild progenitors, and translocations and substitution lines carrying alien segments are promising to augment the genetic diversity in bread wheat cultivars.

In recent past, several alien substitution and translocation lines of wheat (*Triticum aestivum* L.) have been established to enhance the genetic diversity for yield and other desirable traits of the alien donors (Rabinovich, 1998; Ko *et al.,* 2002). The first such hybrid was between wheat and rye (Mujeeb-Kazi and Rajaram, 2002). In the homozygous translocation 1BL.1RS, the long arm of the chromosome 1B (1BL) of *Triticum* genus and

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short arm of *S. cereale* L. chromosome 1R (1RS) are involved (Mujeeb-Kazi *et al.,* 1996) designated as T1BL.1RS. Several advantages are associated with the presence of the rye arm including high yield, stability, and wide adaptability of wheat germplasm. Besides this superiority in grain yield, aerial biomass, kernel weight, and spike fertility have also been attributed to the presence of this translocation in the wheat genotypes (Mujeeb-Kazi *et al.,* 1996; Villareal *et al.,* 1997). In addition, several important biotic resistances such as *Puccinia recondita* (leaf rust), *P. striiformis* (yellow rust), *P. graminis* (stem rust), and *Erysiphe graminis* (downy mildew) have been associated with this short (1R) chromosome arm of rye. These genes are designated as *Lr26, Yr9, Sr31* and *Pm8*, respectively (William and Mujeeb-Kazi, 1993).

Wheats with the 1BL.1RS translocation were described in depth by Mettin *et al.* (1973) and Zeller (1973) with their practical implications presented. This translocation was found in winter wheat cultivar 'Kavkaz' (Trethowan and Mujeeb-Kazi, 2008). The presence of the translocation in "Veery" wheats was diagnosed by Merker (1982), using the Giemsa C-banding diagnostics. Also, Mujeeb-Kazi (1982) independently identified the translocation in various "Veery" derived CIMMYT spring wheat cultivars. Mirzaghaderi *et al.* (2011) studied the distribution of T1BL.1RS in 33 Iranian winter and spring bread wheat cultivars, using mitotic and genomic *in situ* hybridization analyses, verified in 4 cultivars. Recently, Tabibzadeh *et al.* (2013) examined the presence of both T1BL.1RS and T1AL.1RS translocations in 44 Iranian wheat cultivars (29 bread and 15 *durum*), using SDS-PAGE and 3 DNA markers, identified in 5 bread wheats (Atrak, Dez, Falat, Rasul and Moghan3) and not in durum wheats. No T1AL.1RS translocation was detected in Iranian wheat backgrounds in that report. The large scale utilization of these translocated wheats has been initiated and the advantages of such germplasm are documented (Rajaram *et al.,* 1983). Villareal *et al.* (1998) determined the effect of T1BL.1RS chromosome on grain yield and its components and suggested the use of T1BL.1RS wheats in improving the agronomic traits. Kumlay *et al.* (2003) determined the contribution of chromosome arms of group 1 in wheat and rye individually. The effect of T1BL.1RS was investigated on wheat drought (Hoffmann, 2008) or salinity tolerance (Mirzaghaderi *et al.,* 2011).

The global significance of 1RS in wheat breeding programs has been well-reviewed. Baum and Appels (1991) called chromosome 1R 'one of the most widely utilized sources of alien chromatin in wheat cultivars' (Berzonsky and Francki, 1999). This alien segment has significantly contributed to enhance the agronomic performance, particularly grain yield and environmental stability. Hence, wheat breeders worldwide have used the T1BL.1RS germplasms in their breeding programs (Villareal *et al.,* 1997). To use the T1BL.1RS germplasms for wheat improvement, complete characterization of the introgressed segment, determination of the wheat chromosome arm involved in translocation, source of rye chromatin, and the amount of 1RS chromatin introduced are necessary. Cytogenetics, molecular, and biochemical technologies have been reported which enable such a characterization of 1RS in wheat (Berzonsky and Francki, 1999). It is hence crucial that native wheats to Pakistan should be diagnosed for this exchanged genetic material. Hence, the present study was designed to analyze the presence of T1BL.1RS translocation in Pakistani wheat cultivars and candidate lines for future cultivars in the country.

MATERIALS AND METHODS

Plant Material

The experimental material consisted of two sets of germplasm. The first group comprised of the 40 Pakistani elite wheat cultivars (Table 1), whereas the second group comprised of 62 entries of National Uniform Wheat Yield Trial (NUWYT)

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Table 1. A summary of cytological, biochemical, and molecular analysis for the detection of 1BL.1RS translocation in Pakistani wheat cultivars and entries of National Uniform Wheat Yield Trails (NUWYT, 2008-2009 and 2009-2010).

Sr. No	Cultivar/Line	Parentage	Cytological validation (No. of satellites)[a]	C-banding diagnostics [b]	Molecular diagnostics [c]	Glu-B3j[e]
		Pakistanian wheat cultivars				
1	Lasani-2008	LUAN/Kohistan-97	6B,6B,1B,1B	1RS -ve	1RS -ve	1RS -ve
2	Punjnad-88	K4500.2/BJY	6B,6B	1RS +ve	1RS +ve	1RS +ve
3	Miraj-2008	Sparrow/Inia//V.7394/WL711/13/BAU‘S’	6B,6B,1B,1B	1RS -ve	1RS -ve	1RS -ve
4	Kiran-95		6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
5	Farid-2006	PT‘S’/3/TOB/LFN/BB/4/BB/HD-832-5//ON/5/G-V/ALD‘S’//HPO	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
6	Bhittai	VEE/TRAP//Soghat-90	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
7	AS-2002	KHP/D31708//CM74A370/3/Ciano79/4/RL6043/*4NAC	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
8	Faislabad-2008	PBW65/2*Pastor	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
9	Khirman	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
10	Pirsabak-05	Munia/CHTO//Amsel	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
11	Momal-2002	BUC‘S’/4/TZPP/IRN46	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
12	Zarghoon-79	CC/Inia/3/TOB/CTFN//BB/4/7C	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
13	Saleem-2000	Cham-6//Kite/PGO	6B,6B	1RS +ve	1RS +ve	N/A
14	Marvi-2000	CMH-77A917/PKV 1600//RL6010/6*SKA	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
15	Shafaq-2006	V 81094(LU 26/HD 21790/ 2* Inqalab 91)	6B,6B,1B,1B	1RS -ve	1RS -ve	1RS -ve
16	Chakwal-50	Attila/3/HUI/CARC//CHEN/CHTO/4/Attila	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
17	Sehar-2006	CHILL/2* Star/4/BOW//BUC/PVN/3/2*VEE#10	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
18	TD-1	MAI’S’ X NORTENO65 X H68	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
19	Imdad-2005	N/A	6B,6B	1RS +ve	1RS +ve	N/A
20	Pak-81	KVZ//BUHO//KAL/BB	6B,6B	1RS +ve	1RS +ve	1RS +ve
21	Shalimar-88	PB81/HD2182/PB81	6B,6B	1RS +ve	1RS +ve	1RS +ve
22	Pavon	VCM//CNO/7C/3/KAL/BB	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
23	Auqab-2000	Crow‘s’/NAC//BOW‘S’	6B,6B,1B,1B	1RS -ve	1RS -ve	1RS -ve
24	Tatara	JUP/ALD‘S’//KLT’S	6B,6B	1RS +ve	1RS +ve	N/A
25	Sarsabz	M20/79	6B,6B,1B,1B	1RS -ve	1RS -ve	1RS -ve
26	Chakwal-97	BUC’S’/FCT‘S’	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
27	Margalla-99	Opata/BOW‘S’	6B,6B,1B,1B	1RS -ve	1RS -ve	1RS -ve
28	Inqalab-91	WL 711/Crow‘s’	6B,6B,1B,1B	1RS -ve	1RS -ve	1RS -ve
29	Wafaq-2001	Opata/Rayon//Kauz	6B,6B	1RS +ve	1RS +ve	N/A
30	Blue Silver	1154-388/AN/3/YT54/N10B//LR64	6B,6B,1B,1B	1RS -ve	1RS -ve	1RS -ve
31	Tandojam-83	TZPP/PL/7C	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
32	GA-2002	DWL 5023/S N B// SNB	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
33	Pirsabak-85	KVZ/BUHO/KAL/BB	6B,6B	1RS +ve	1RS +ve	1RS +ve
34	Chakwal-86	Fln/ACS/ANA	6B,6B	1RS +ve	1RS +ve	N/A
35	LU-26	Blue Silver/Khushal	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
36	Pasban-90	Inia F 66/A.Distchum//Inia66/3/GEN	6B,6B	1RS +ve	1RS +ve	1RS +ve
37	Bhakkar-2002	P20102/PIMA/SKA/3/TTR‘S’/BOW‘S’,Pb.23826-D-1a-1a-1t-1t-0t	6B,6B,1B,1B	1RS -ve	1RS -ve	1RS -ve
38	Rohtas-90	Inia F 66/A.Distchum//Inia66/3/GEN	6B,6B	1RS +ve	1RS +ve	1RS +ve
39	Fakhr-e-Sarhad	PFAU‘S’/Seri//BOW‘S’	6B,6B	1RS +ve	1RS +ve	1RS +ve
40	Zardana	CNO S/8156 TOB 66 CNO6-PVN	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A

[a] The presence of 2 satellites (6B, 6B) shows the presence of translocation, and the presence of 4 satelllites (6B, 6B, 1B, 1B) shows its absence; [b] 1RS+ve (positive) shows the presence of heterochromatin bands of rye, 1RS –ve shows its absence; [c] 1RS+ve (positive) shows the presence of translocation, 1RS -ve shows the its absence, [d] 1RS+ve (positive) shows the presence of *Glu-B3j*, a low molecular weight subunit encoded by rye chromatin specific for translocation, 1RS –ve (negative) shows its absence. **Table 1** Continued

Table 1 continued.

Sr. No	Cultivar/Line	Parentage	Cytological validation (No. of satellites)[a]	C-banding diagnostics [b]	Molecular diagnostics [c]	Glu-B3j[e]
		Pakistanian wheat cultivars				
41	NR-358	PFAU/Weaver*2//Kiritati CGSS01B00076T-099Y-099M-099B-75Y-0B-01D	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
42	PR-98	CMH84.3379/CMH78.578//Milan CMSS93Y006285-7Y-010Y-010M-010Y-10M-0Y-3KBY-0KBY	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
43	NR-360	PFAU/Seri.1B//AMAD/3/Waxwing CGSS02Y00153S-099M-099Y-099M-46Y-0B-01D	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
44	SN-151	Kambara-1 CGSS9500016F-099Y-099B-099Y-099B-15Y-0B-0SY	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
45	04FJS35	PASTOR//HXL7573/2*BAU CMSS97M00306S-0P-95Y-90M-010Y	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
46	AZRC-2008-1	Tracha's//CMH76-252/Pvn's ICW93-0065-6AP-0L-3AP-0L-1AP-0AP	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
47	PR-99	Hamam-4/Star"S"/Liz 0F-0K-2F-0K	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
48	KT-4	Altar84/*Ae.squarrosa*219//SER CMBW91Y00892S-8Y-11KBY-2KBY-010M-9Y-3M-0Y-0SY	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
49	V-05003	Karvan2/4/Burgus/Sort12-13//Kal/BB/3/Pak81	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
50	NRL-0320	FRET 2 CGSS96Y00146T-099B-099Y-099B-16Y-0B-0SY	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
51	5C011	Skauz/BAV92 CMSS96M03611S-1M-010SY-010M-010SY-8M-0Y	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
52	NUWYT-1	N/A	6B,6B	1RS +ve	1RS +ve	N/A
53	NUWYT-2	N/A	6B,6B	1RS +ve	1RS +ve	N/A
54	NUWYT-3	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
55	NUWYT-4	N/A	6B,6B	1RS +ve	1RS +ve	N/A
56	NUWYT-5	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
57	NUWYT-6	N/A	6B,6B	1RS +ve	1RS +ve	N/A
58	NUWYT-7	N/A	6B,6B	1RS +ve	1RS +ve	N/A
59	NUWYT-8	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
60	NUWYT-9	N/A	6B,6B	1RS +ve	1RS +ve	N/A
61	NUWYT-10	N/A	6B,6B	1RS +ve	1RS +ve	N/A
62	NUWYT-11	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
63	NUWYT-12	N/A	6B,6B	1RS +ve	1RS +ve	N/A
		National Uniform Wheat Yield Trials (NUWYT) irrigated				
64	DN-62	SW89.5181/Kauz CMSS93B00824S-24Y-010M-010Y-010M-9Y-0M-0HTY	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
65	V-04178	Shalimar88/90A-204//MH97	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
66	SM-07018	Shalimar-88/Atilla//MH97	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
67	22-03	Snb(s')//Kea(s')/Snb(s')	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
68	B-07/Bkhtwr	LFN/1158.57//Prl/3/Hahn/4/Kauz CMBW 89Y1044-0t0PM-8Y-010M-020B-0NPL-0T0Y	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
69	V-05082	Chenab2000/ Inqalab-91	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
70	PR-90	CNDO/R143//Ente/Mexi-213/ CMSS93Bo1824M-040Y-73Y-010M-010Y-010M-10Y-0M	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A

[a] The presence of 2 satellites (6B, 6B) shows the presence of translocation, and the presence of 4 satelllites (6B, 6B, 1B, 1B) shows its absence; [b] 1RS+ve (positive) shows the presence of heterochromatin bands of rye, 1RS –ve shows its absence; [c] 1RS+ve (positive) shows the presence of translocation, 1RS -ve shows the its absence, [d] 1RS+ve (positive) shows the presence of *Glu-B3j*, a low molecular weight subunit encoded by rye chromatin specific for translocation, 1RS –ve (negative) shows its absence. **Table 1** Continued

Table 1 continued.

Sr. No	Cultivar/Line	Parentage	Cytological validation (No. of satellites)[a]	C-banding diagnostics [b]	Molecular diagnostics [c]	Glu-B3j[e]
		Pakistanian wheat cultivars				
71	ZAS70	Inqalab 91*2/Tukuru CGSS99B00015F-099Y-099M-099Y-099M-31Y-0B	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
72	33010	KT/Bage//Fnu/3/Chakwal-86 BR.4457-1B-5B-3B-0B	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
73	AUP-4008	Gen*2//Buc/Flk/3/Buchin CMSS96M03098S-12M-010SY-010M-010SY-3M-0Y	6B,6B	1RS +ve	1RS +ve	N/A
74	NIA-8/7	SHA4/Weaver//Skauz*2/SRMA	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
75	V-05066	Amsel/Attila// Inqalab-91/Pew'S'	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
76	CT-03457	Attila*2/Yaco CGSS96B00134F-099B-028Y-099M-4Y-0B	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
77	66284	Inqalab-91/CB-271	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
78	SD-4085/3	Sarsabz/Sunco*2	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
79	NR-356	Oasis/Skauz//4*BC/3/2*Pastor CMSS00Y01881T-050M-030Y-030M-030WGY-33M-0Y-01D	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
80	9268	7012/PBW-222	6B,6B	1RS +ve	1RS +ve	N/A
81	V-05BT006	Maya/Mon'S'//Hork/Fsd85 Iotech-0R4-1R1-2R7-3RK-0R	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
82	V-04022	Inqalab-91/3/Crow/Nac//Bow'S'	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
83	NUWYT-13	N/A	6B,6B	1RS +ve	1RS +ve	N/A
84	NUWYT-14	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
85	NUWYT-15	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
86	NUWYT-16	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
87	NUWYT-17	N/A	6B,6B	1RS +ve	1RS +ve	N/A
88	NUWYT-18	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
89	NUWYT-19	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
90	NUWYT-20	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
91	NUWYT-21	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
92	NUWYT-22	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
93	NUWYT-23	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
94	NUWYT-24	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
95	NUWYT-25	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
96	NUWYT-26	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
97	NUWYT-27	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
98	NUWYT-28	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
99	NUWYT-29	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
100	NUWYT-30	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
101	NUWYT-31	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A
102	NUWYT-32	N/A	6B,6B,1B,1B	1RS -ve	1RS -ve	N/A

[a] The presence of 2 satellites (6B, 6B) shows the presence of translocation, and the presence of 4 satelllites (6B, 6B, 1B, 1B) shows its absence; [b] 1RS+ve (positive) shows the presence of heterochromatin bands of rye, 1RS –ve shows its absence; [c] 1RS+ve (positive) shows the presence of translocation, 1RS -ve shows the its absence, [d] 1RS+ve (positive) shows the presence of *Glu-B3j*, a low molecular weight subunit encoded by rye chromatin specific for translocation, 1RS –ve (negative) shows its absence.

nursery of 2 crop seasons 2008-2009 and 2009-2010 for both rainfed and irrigated categories. These categories of rainfed and irrigated denote the two major areas of wheat production based on the available source of water. Among the 40 elite cultivars germplasm, wheat genotypes were randomly selected for the determination of the 1BL.1RS translocation.

Cytological Analysis

Conventional cytological techniques that differentiated the satellited 1B and 6B chromosomes were employed (Mujeeb-Kazi *et al.,* 1994). Constitutive heterochromatin Banding (C-Banding) and fluorescent *in situ* hybridization (FISH) was also performed (Jahan *et al.,* 1990; Mujeeb-Kazi *et al.,* 1994, 1996; Mirzaghaderi *et al.,* 2010).

Mitotic Analysis

Seeds of each entry were germinated in petri plates having moist filter papers. After two days, root tips were collected and pre-treated for three hours with colchicines, 8-hydroxyquinoline, and dimethylsulphoxide (DMSO) and fixed by staining in 2% (w/v) aceto-orcein prepared in 45% (v/v) acetic acid. The samples were refrigerated at 4°C until 2 days prior to mitotic preparations. Softening of the tissues was carried out by heating the root tips in 45% acetic acid. The apex was cut off and tip longitudinally slit by a fine needle, while the root was held by forceps on the clean glass slide. With the aid of an arrow-head needle, the cells were excised onto the slide; a drop of 45% acetic acid was added. Mitotic counts of each sample were performed on metaphase cells, over 2 cells per sample were counted for the verification of chromosome number and the identification of satellites to differentiate between the translocated and non-translocated genotypes. Slides were made permanent by removing the cover slip in liquid nitrogen, rapidly dehydrating in absolute alcohol and mounting in Canda balsam (Mujeeb-Kazi *et al.,* 1994).

Constitutive Heterochromatin Banding (C-Banding) and Fluorescent in situ Hybridization (FISH)

Root tips were collected from germinated seeds of the germplasm and pre-treated with colchicine for a minimum of 3 hours 15 minuntes and were then transferred to 0.2% (w/v) aceto carmine and left overnight in the refrigerator. Squashing of these root tips was done and they were put in the ultra freezer at -80°C for 15 minutes to remove the cover slip. Staining was performed according to the procedure desribed by Mujeeb-Kazi *et al.* (1994). After staining, standard banding patterns of 1RS and 1BL chromosomes were observed under the microscope for identification of translocated and non-translocated genotypes. Squashed root tips were also subjected to *in situ* hybridization according to the protocol of Mujeeb-Kazi *et al.* (1996). Wheat DNA was used as the blocking DNA and rye was the labeled DNA.

Molecular Analysis

DNA Extraction

The seeds of the germplasm were sown under controlled conditions in a growth chamber. Young leaves of the 10-days-old seedlings were clipped for DNA extraction. DNA was extracted using the procedure described by Weining and Langridge (1991). The DNA was stored at 4°C for future use. Dilutions were prepared using the double distilled, deionized or the autoclaved water in the ratio of 1:5 to be used in Polymerase Chain Reaction (PCR).

Molecular Marker

The 1RS specific marker NOR (F: GCATGTAGCGACTAACTCATC, R:

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CCCAGTTTTCCATGTCGC; 400, 700 and 800 bp diagnostic) was used for the identification of translocation (Koebner, 1995).

Polymerase Chain Reaction

Molecular analysis was carried out using SSR primer linked to the rye chromosome (Weining and Langridge, 1991). PCR was performed in a Gene Amp (R) PCR System 9700 Thermo-cycler. PCR reaction was carried out in 25 µL reaction mixture consisting of final concentration of 0.4 uM each primer, 0.2 mM each of dCTP, dGTP, dTTP and dATP (Sigma Chemical Co., St. Louis, MO, USA), 2.5 mM $MgCl_2$, 1X PCR Buffer, 1 unit μL^{-1} of Taq DNA polymerase (Promega Madison WI, USA), and 2 ng μL^{-1} genomic DNA. After 5 minutes of denaturation at 94°C, amplifications were programmed for 40 consecutive cycles each consisting of 30 seconds at 94°C, 30 seconds at 50-60°C (depending on the individual primer), 30 seconds at 72°C and followed by a 10 minutes extension step at 72°C. After electrophoresis of PCR products, gels were visualized by Ethidium Bromide staining under the UV light chamber and observed, using the computer program UVIPhotoMW.

Low Molecular Weight Glutenin Subunits Analysis

Identification of B-genome encoded low molecular weight (LMW) glutenin allele, *Glu-B3j,* was used as biochemical indicator for the presence of T1B.1R translocation (He *et al.,* 2005). Glutenins were extracted and separated on SDS-PAGE following the protocol of Bibi *et al.* (2012).

RESULTS

Results of the mitotic analysis and C-banding are displayed in Table 1 and figure1. In 12 (30%) out of 40 wheat cultivars, only one pair of satellites on 6B chromosomes was observed, while the remaining 28 (70%) cultivars had 2 pairs of satellites on chromosomes 1B and 6B, respectively. The satellites on 5D chromosome appeared rarely. Cultivars carrying T1BL.1RS translocation were identified as follows: Punjnad-88, Chakwal-86, Saleem-2000, Imdad-2005, Pak-81, Shalimar-88, Tatara, Wafaq-2001, Pirsabak-85, Pasban-90, Rohtas-90 and Fakhr-e-Sarhad. In the NUWYT group, 10 out of 23 entries of rainfed category were identified as carrying T1BL.1RS translocation. Four out of 39 genotypes were identified as translocated in the irrigated category of both crop seasons. These genotypes showed one pair of prominent satellite chromosomes, presumably lacking 1BS and containing 1RS. The 1RS satellite in the T1BL.1RS does not express, hence, wheats with 2 satellites have chromosome 6B and the translocation. The C-banding analysis similarly identified the same genotypes as carrying 1BL.1RS translocation based on the prominent heterochromatin banding.

Results of the 1RS specific low molecular weight subunit (LMW-GS) and molecular marker are summarized in Table 1 and displayed in Figures 2 and 3, respectively. The 1RS specific marker amplifies the rye chromosome. The presence of the band indicates the translocation and null allele shows the lack of translocation. The marker, in this study, amplified the scorable bands ranging from 300 to 800 bp. The maximum number of bands observed was 3 while the minimum number of bands was 2. A total of 12 out of 40 Pakistani cultivars were amplified by this marker. In the total NUWYT entries of both crop seasons 2008-2009 and 2009-2010, 14 possessed translocation with the amplification of 400, 700, and 800 bp diagnostic band. The genotypes showing amplification carried the 1BL.1RS translocation. For further authentication, low molecular weight glutenin subunit analysis was also done. This analysis revealed 7 cultivars as translocated out of 16 cultivars, while the remaining 9 were found to be non-tanslocated. The translocated cultivars expressed the low molecular weight subunit *Glu-B3j,* while the non-translocated cultivars did not express this subunit. The cultivars found to be translocated included Punjnad-88, Pak-81, Shalimar-88, Tatara, Pirsabak-85, Pasban-90, Rohtas-90 and Fakhr-e-Sarhad; the remaining cultivars were non-translocated.

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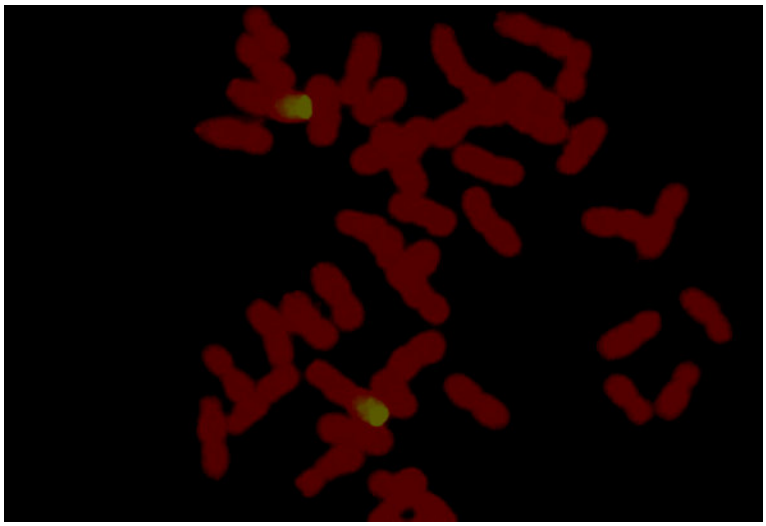

Figure 1. Fluorescent *in situ* hybridization (wheat DNA for blocking and rye DNA is labeled) somatic cell from a wheat-rye translocation genotype. Wheat chromosomes are reddish in color and the yellowish short arm belongs to 1RS.

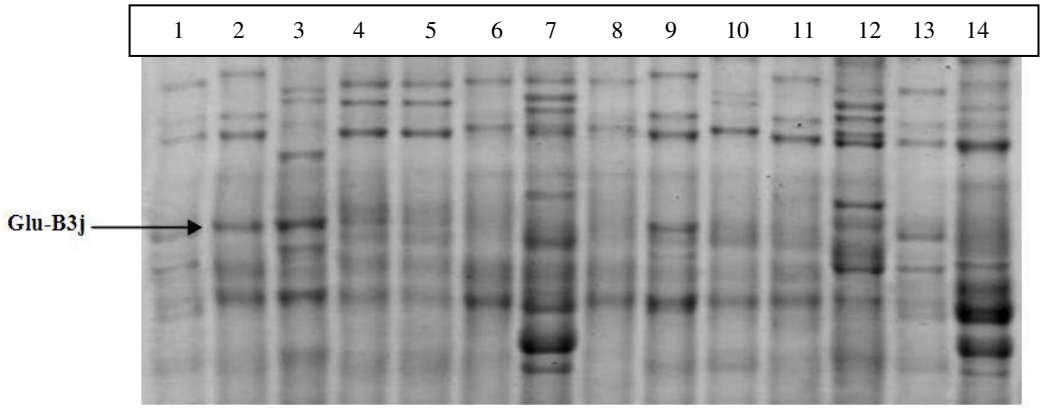

Figure 2. Presence of *Glu-B3j* allele as biochemical marker of 1B.1R translocation in wheat. Arrow shows presence of marker in 1 (From left): Lasani, 2: Pak81, 3: Shalimar88, 9: Shafaq-2006 and 13: Blue Silver, while it is absent in lanes; 4: Rohtas-90; 5: Pirsabak-05; 6: Moomal-2002, 7: Auqab-2000; 8: Bakhar-2002; 10: Auqab-2000; 11: Sarsabz; 12: Pirsabak-85, 14: Inquilab-91**.**

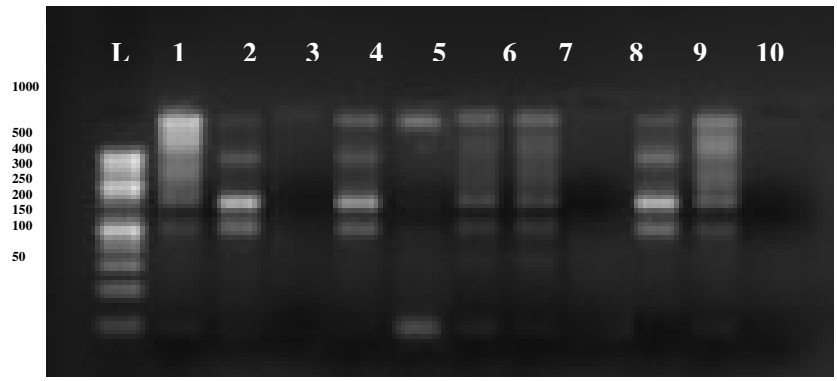

Figure 3. Marker analysis of NUWYT (1-10) entries in 2009-10 crop season by primer *NOR* (400, 700 and 800 bp diagnostic). Rye segment present in 1: NUWYT-1; 2: NUWYT-2; 4: NUWYT-4; 6: NUWYT-6; 7: NUWYT-7; 9: NUWYT-9; 10: NUWYT-10, and absent in NUWYT-3; NUWYT-5, NUWYT-8.

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DISCUSSION

A variety of approaches are being used to identify T1BL.1RS chromosome translocations in bread wheat including conventional cytology (Zeller, 1973), biochemical analysis (Koebner and Shepherd 1986; Landjeva *et al.,* 2006), chromosome N and, C-banding (Rayburn and Carver, 1988; Mirzaghaderi *et al.,* 2010), PCR with specific primers (Koebner, 1995; Weng *et al.,* 2007; Tabibzadeh *et al.,* 2013), and PCR-ELISA (Zuniga *et al.,* 2008). The mitotic analysis for the diagnosis of translocation is based on the identification of satellites in the chromosomes of somatic cells. The satellite or secondary constriction of 1RS is not expressed in a wheat genetic background (Merker, 1982). In lines lacking a T1BL.1RS translocation, somatic cells usually have 4 chromosomes with prominent satellites associated with their short arms: two 1BS and two 6BS chromosomes. Because 1RS replaces 1BS in a Robertsonian 1BL.1RS translocation, the detection of only 2 satellite chromosomes in somatic cells of high quality preparations gives a quick and initial indication of the 1BL.1RS translocation. Using the satellite chromosomes counting, we identified 12 out of 40 Pakistani wheat cultivars carrying rye translocation. This technique is easy, inexpensive, and convenient to diagnose the 1RS rye translocations in bread wheat. However, handling and preparation of slides in this technique becomes a limiting factor affecting result efficiency. This technique is based on the mitotic counts of satellite chromosomes and has been carried out previously for the determination of 1BL.1RS rye translocation in wheat genotypes (Mettin *et al.,* 1973; Mujeeb-Kazi and Miranda 1985; Jahan *et al.,* 1990; Mujeeb-Kazi *et al.,* 1994; Berzonsky and Francki, 1999; Mirzaghaderi *et al.,* 2011; Tabibzadeh *et al.,* 2013). Apart from mitotic studies, we used C-banding, FISH, low molecular weight subunit *Glu-B3j*, and molecular marker. The integrated results of all these techniques complemented each other.

The presence of rye translocation (1BL.1RS) in Pakistani wheat cultivars and candidate lines is attributed to the use of wheat germplasm distributed by CIMMYT. In order to broaden its genetic base for biotic and abiotic stresses and agronomic performance, the 1RS rye translocations have been introgressed in bread wheat germplasm. The chromosome arm 1RS harbours genes conferring resistance to biotic stresses (Heun and Fischbeck, 1987; Singh *et al.*, 1990; Mcintosh *et al*., 1993). Besides these resistance genes, 1RS has genetic factors for wide adaptation and tolerance to abiotic stresses (Rajaram *et al.,* 1983; Villareal *et al.,* 1994) and also contributes towards higher grain yield (Schlegel and Meinel 1994; Caver and Rayburn 1994; Moreno-Sevilla *et al.,* 1995; Villareal *et al.,* 1991, 1997, 1998). In wheat genotypes with T1BL.1RS, breakdown of resistance to leaf rust due to *Lr26* and that of powdery mildew due to *Pm8* in Europe and Mexico (Zeller and Hsam 1984; Bennett 1984; Lutz *et al.,* 1992; Villareal *et al.,* 1998) and *Sr31* due to the evolution of the devastating Ug-99 caused the wheat genetic base narrower. Additionally, deleterious effects of the rye translocation on bread making quality have urged to search for other rye chromosomes with broader genetic diversity. Notables are 1AL.1RS and 15 other available translocations (Jiang *et al.,* 1994; Friebe *et al.,* 1996; Tabibzadeh *et al.,* 2013). It is, therefore, prerequisite in Pakistan and other wheat growing countries to use rye translocations other than T1BL.1RS to ensure long lasting control against the biotic/abiotic stresses and to attain higher grain yield output.

In conclusion, we have generated valuable information about Pakistani wheat cultivars and the entries of NUWYT for the two crop seasons 2008-2009 and 2009-2010. The results showed that the percentage of entries possessing T1BL.1RS translocation increased from 6.7% in 2008-09 to 31.2% in 2009-2010. This 4.6-fold increase is desirable as many advantages are associated with this translocation. However, some quality concerns associated with the short arm of rye exist such as diminished mixing tolerance, dough, stickiness, reduced loaf volume, and poor crumb grain when compared with wheats, but still the advantages are more, making the 1B.1R translocation desirable to be used in breeding programs as in India (Kumar *et al.,*. 2003) and in Iran (Mirzaghader *et al.,* 2011; Tabibzadeh *et al.,* 2013), both neighboring countries to Pakistan. The future strategies to exploit such sources to get more benefit with this sort of novel germplasm continue to receive encouragement. The emphasis has also been placed on the utilization of translocations other

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than 1BL.1RS to enhance the genetic base of existing wheat cultivars, because the genes residing on this translocation have broken down. An in-depth revision (Mujeeb-Kazi *et al.* 2013; personal communication) plus novel strategies of exploiting and producing wheat/alien translocations has emerged. We suggest that the future course may diversify the wheat genetic base swiftly since leading cultivars with T1BL.1RS are all structured around 4 uniform cultivars in all the pedigrees of such cultivars globally grown, rendering all with a narrow genetic base and a threat for yield performance durability (Kavkaz, Buho, Kalyansona, and Bluebird).

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ارزیابی ذخایر ژنتیکی نخبه گندم پاکستانی برای جا به جایی کروموزومیT1BL.1RS

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چکیده

کروموزومIRS چاودار(*Secale cereal*) حامل چندین ژن از جمله *Lr26، Yr9،Sr31* و*Pm8* میباشد که خاصیت مقاومت به امراض و تنش های غیر زیستی القا می کنند. انتقال اطلاعات ژنتیکی بازوی کوتاه کروموزم IR چاودار کمک فراوانی به تنوع ژنتیکی در گندم کرده است. استفاده از این جا به جایی کروموزوم در برنامه های بهنژادی نیازمند شناسایی ذخایر ژنتیکی گندم است که دارای جا به جایی کروموزوم گندم-کروموزوم خارجی هستند. پژوهش حاضربرای غربال کردن مجموعه ای از ۱۰۲ کالتیوار و لاین نامزد (رگه) گندم پاکستانی برای شناسایی جا به جایی کروموزمی T1BL.1RSچاودار با استفاده از روش های یاخته شناسی، بیوشیمی، و ملکولی طراحی واجرا شد. نتایج آشکار کرد که ازمیان ۴۰ کالتیوار گندم ۱۲ کالتیوار دارای این انتقال اطلاعات ژنتیکی خارجی بودند. همچنین، در آزمون های معروف به "آزمون های ملی و یکنواخت عملکرد گندم" (NUWYT) مشخص شد که تعداد ۱۰ ورودی (entry) از میان ۲۳ ورودی گروه دیم حامل جابجایی کروموزمی 1BL.1RS بودند در حالی که در گروه آبیاری شده در هر دو فصل اجرای NUWYT ،از میان ۳۹ ژرم پلاسم فقط ۴ مورد آن را داشتند. این اطلاعات ارزشمند می تواند در برنامه های اصلاح نباتات برای تولید ژرم پلاسم های دارای جا به جایی T1BL.1RSبه منظور افزودن تغییرات ژنتیکی در کالتیوارهای محلی و نیز جلوگیری از چیرگی وکثرت نامزدهای T1BL.1RS مفید باشد.

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